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TI Role of gastrointestinal microflora in the mineral absorption of young adult mice
AU Yoshida, Tsutomu; Oowada, Tsutomu; Ozaki, Akira; Mizutani, Takeo
CS Fac. Nutr., Kagawa Nutr. Univ., Sakado, 350-02, Japan
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actually less Ca is absorbed in GB mice

happened to be not good ref.

not good or bad

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Note

Role of Gastrointestinal Microflora in the Mineral Absorption of Young Adult Mice

Tsutomu YOSHIDA,[†] Tsutomu OOWADA,* Akira OZAKI,* and Takeo MIZUTANI*

Faculty of Nutrition, Kagawa Nutrition University, Sakado-shi, Saitama 350–02, Japan
** Laboratory Animal Research Center, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351–01, Japan*
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A comparison between germfree (GF) and gnotobiotic (GB) mice, inoculated with *Bacteroides vulgatus*, *Eubacterium aerofaciens*, *Bifidobacterium longum*, *Enterococcus faecalis*, *Escherichia coli*, and *Clostridium perfringens*, revealed that the GB mice suffered no deleterious effect on the apparent absorption ratios of Ca and P, and showed a higher apparent absorption ratio of Mg.

Our previous studies[1,2] on growing mice have shown that conventional conditions resulted in lower apparent absorption (=absorption) ratios of Ca, P, and Mg than those in GF counterparts. This means that gastrointestinal microflora had a deleterious effect on the absorption of these minerals. However, there are no reports on the kinds of gastrointestinal microflora which affect mineral absorption. Therefore, the studies reported here were made on young adult mice to elucidate the effect on Ca, P, and Mg absorption of inoculating with 6 species of intestinal bacteria, these being obtained from the human intestine and being well established in the intestine of ex-GF mice.

Male GF and GB mice from a CF#1 inbred colony were used in this investigation. The mice were maintained in plastic rearing cages (six mice per cage) and kept in Trexler flexible plastic isolators in the animal room at a temperature of 23 ± 1°C and relative humidity of 50 ± 10% with a 12 h light/dark cycle. The mice were fed on a practical diet for specially bred mice and rats from Oriental Yeast Co., Tokyo (CMF diet). The diet was sterilized by 50 kGy of irradiation with ^{60}Co, and deionized distilled water was autoclaved for 1 h at 125°C. The diet and drinking water were provided *ad libitum* throughout the experimental period.

GB mice were obtained by orally inoculating GF mice at the age of 6 wk with 6 species of bacteria obtained from the human intestine: *Bacteroides vulgatus* XI-87 (ATCC8482), *Eubacterium aerofaciens* 151, *Bifidobacterium longum* E-194B, *Enterococcus faecalis* 1–12, *Escherichia coli* 123, and *Clostridium perfringens* MAC521. The log bacterial counts per g of fresh feces from the GB mice at 8 wk of age were 10.6, 10.4, 10.3, 10.1, 9.2, and 7.8, respectively. These intestinal microflora were well established in the same order as that in the human intestine.[3]

The body weight of each mouse, and the diet consumption and fecal excretion for each group of 6 mice were measured weekly for 4 wk from 14 wk of age. The feces was stored at −20°C and dried at 30°C in a vacuum. The diet and dried feces were finely ground, and wet-ashed with HNO_3–$HClO_4$ (1 : 5, v/v). An atomic absorption spectophotometer (Hitachi 170-50A) was used for determining Ca and Mg, and P was analyzed by Chen's method.[4] Student's *t*-test was employed for statistical evaluation, differences being considered to be significant with p-values < 0.05.

The analytical contents (day matter basis) of Ca, P, and Mg in the diet were 1.24%, 0.87%, and 0.28%, respectively.

As shown in Table I, the initial body weights (14 wk of age) of the GF and GB mice were 40.5 g and 39.2 g, respectively, and no significant difference was found between the GF and GB mice. However, the final body weights (18 wk of age) of the GF and GB mice at sacrifice were 43.8 g and 40.9 g, respectively, showing a greater difference than that of the initial body weight between the two groups, although the difference was not significant. This result is consistent with that of a previous study[1] on a comparison between GF and conventional mice.

The average 4 wk contents of fecal moisture from the GF and GB mice were 62.3% and 47.5%, respectively, a significant

Table I. Initial and Final Body Weights (g per mouse) and Moisture Content (%) of Fresh Feces for a 4 Week Period in Germfree and Gnotobiotic Mice

	Initial body weight* (g)	Final body weight* (g)	Moisture in fresh feces** (%)
GF mice[≠]	40.5	43.8	62.3
	±1.8	±2.6	±1.0
GB mice[≠]	39.2	40.9	47.5[a]
	±3.6	±2.8	±1.4

Data show the mean values ± standard deviations for 6 mice* or 4 weeks**.
[≠] GF mice = germfree mice; GB mice = gnotobiotic mice.
[a] Significantly different from the corresponding value for GF mice[≠] at $p < 0.01$.

Table II. Daily Intake, Fecal Excretion and Apparent Absorption of the Diet, Ca, P, and Mg in Germfree and Gnotobiotic Mice (per mouse per day)

		Intake (mg)	Fecal excretion (mg)	Apparent absorption (mg)	Absorption ratio* (%)
Diet	GF[≠]	4020	1420	2610	64.8
(dry matter)		±130	±40	±100	±0.4
	GB[≠]	3620[a]	1050[a]	2580	71.2[a]
		±150	±20	±140	±0.9
Ca	GF	49.9	43.2	6.7	13
		±1.7	±1.5	±0.8	±1
	GB	44.9[a]	39.4[a]	5.6	12
		±1.9	±1.2	±1.8	±4
P	GF	35.1	26.6	8.5	24
		±1.2	±1.0	±0.8	±2
	GB	31.6[a]	25.2	6.4[b]	20
		±1.3	±0.7	±1.2	±3
Mg	GF	11.2	7.8	3.4	30
		±0.4	±0.2	±0.2	±1
	GB	10.1[a]	6.5[a]	3.6	36[a]
		±0.4	±0.0	±0.4	±2

Data show the mean values ± standard deviations for a 4 week period.
* (Diet intake − fecal excretion)/diet intake.
[≠] GF = germfree mice; GB = gnotobiotic mice.
[a,b] Significantly different from the corresponding value for GF[≠] mice at $p < 0.01$ and $p < 0.05$.

[†] To whom requests for reprints should be addressed.

difference being found between the two groups. The same results were also obtained from experiments on rats[5] and rabbits,[6] showing that feces from conventional animals contained less moisture than those from their GF counterparts.

Table II summarizes the intake, fecal excretion, absorption and absorption ratio of the diet, Ca, P, and Mg in GF and GB mice as averages of 4 wk values from 14 wk of age.

With respect to the diet (dry matter), GB mice had a lower intake and fecal excretion, and thus a similar amount of absorption. Therefore, a higher absorption ratio was obtained for GB mice (71.2%) than for GF mice (64.8%), and this result is in accord with that of our previous experiment,[7] in which higher digestibility of dry matter in the diet by conventional mice than by GF mice was caused by the higher digestibility of the N-free extract. An anorexigenic substance that inhibits spontaneous diet intake has been isolated from the feces of conventional, but not GF, rats and mice[8] including *Eschrichia coli*,[9] and one reason for the lower diet intake of GB mice is associated with this anorexigenic substance in GB feces.

Owing to the lower intake of diet, a significantly lower intake of the three minerals in GB than in GF mice was observed. Moreover, GB mice had a significantly lower fecal excretion of Ca, although the absorption and absorption ratio between GF and GB mice were similar. For P balance, the GB mice had a significantly lower intake and absorption than the GF mice did, and thus the absorption ratio was similar for the GF and GB mice. We hava previously reported that the ratio of Ca and P absorption was lower in conventional mice than in GF mice.[1] Compared to the GF animals, their conventional counterparts had lower levels of intestinal brush border Ca^{2+}-stimulated ATPase and alkaline phosphatase together with mucosal Ca-binding protein, which may have been responsible for the decreased Ca and P absorption.[1] Moreover, conjugated bile acids, which may enhance Ca absorption by GF animals by retaining most in their intestines, are mainly changed to the deconjugated form in conventional animals.[1] Therefore, it is conceivable that these conventional situations might not have occurred from the enteric microflora used in the present experiment.

The data for Mg balance show significantly lower intake and fecal excretion with the GB mice than with the GF mice, and no difference in absorption between the two groups. Thus, a higher absorption ratio was obtained for the GB mice. The present result for GB mice is quite different from that of our previous study,[1] in which a significantly lower ratio of Mg absorption was observed in conventional mice than in GF mice. Therefore, the inoculation of GB mice with 6 kinds of intestinal bacteria in this experiment had no deleterious effects on the absorption of Ca, P, and Mg, but actually had a desirable influence of Mg absorption. We have already observed an appreciable absorption of Mg in the large intestine of rats,[10] where the absorption ratio of Mg was much higher than that of Ca and P absorption. Thus, it is probable that the large intestinal bacteria may have a stronger influence of Mg absorption that on Ca and P absorption.

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